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STUDIES ON THE AGGREGATION OF SOME PEPTIDES RELATED TO AMYLOID b-PEPTIDE. O.M. El-Agnaf, G.B. Irvine, D.J.S. Guthrie, D.M. Walsh. Division of Biochemistry, School of Biology & Biochemistry, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, U.K.

The amyloid β-peptide (Aβ), found deposited in fibril form in characteristic plaques in the brains of patients with Alzheimer's disease, comprises 39-43 amino acids derived from amyloid precursor protein. Observed neurotoxicity of Aβ analogs correlates with the ability of the analog to aggregate in vitro. Since residues 25-35 of Aβ have been implicated in this neurotoxicity, we have studied the aggregative properties of several analogues containing this region. Met^{35} was replaced by Nle to avoid possible problems due to oxidation. The peptides examined were [Nle^{35}]Aβ(25-35)amide, [Nle^{35}]Aβ(23-35)amide, [Nle^{35}]Aβ(21-35)amide and [Nle^{35}]Aβ(19-35)amide. In addition, since an Ala^{21} to Gly mutation has been found in a family with a history of both pre-senile dementia and cerebrovascular amyloidosis, we synthesised [$Gly^{21}Nle^{35}$]Aβ(19-35)amide. All these peptides were soluble in water only after subjecting an aqueous suspension to a short treatment with ultrasound. Upon aging, all the peptides formed assemblies having distinct fibrillar morphologies as observed by electron microscopy of negatively stained samples. The rates at which aggregation occurred increased with increasing peptide length. [$Gly^{21}Nle^{35}$]Aβ(19-35)amide had an enhanced tendency to aggregate relative to [Nle^{35}]Aβ(19-35)amide, whereas full length [Gly^{21}]Aβ has been reported to aggregate in vitro less rapidly than the Ala^{21} peptide (Clements et al., Neurobiology of Aging (1994) 15, S48-S49).

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CHARACTERIZATION OF PURIFIED SUBTILISIN-LIKE PROHORMONE CONVERTASES PC1/3 AND PC2, AND AN ASPARTIC PROTEASE FROM CHROMAFFIN GRANULES. A.V. Azaryan*, M. Schiller*, L. Mende-Mueller+, V.Y.H. Hook. Department of Medicine, University of California, San Diego; *Dept. of Biochemistry, Uniformed Services University, Bethesda, MD;+ Dept. of Biochemistry, Medical College of Wisconsin, Milwaukee, WI.

Three candidate prohormone convertases were purified to apparent homogeneity from bovine adrenal medulla chromaffin granules (CG) by chromatography on ConA-Sepharose, Sephacryl S200, pepstatin A agarose, and immunoaffinity columns. At the Sephacryl S200 step, the main peak eluting at the void volume has been identified as the novel cysteine protease 'prohormone thiol protease.' A secondary peak of activity was identified in this study as PC1/3 and PC2 that belong to the family of subtilisin-related proprotein convertases, and an aspartic protease. The activities of the PC enzymes were assayed with ^{35}S-preproenkephalin (^{35}S-PPE) and Z-Arg-Val-Arg-Arg-MCA (pH optimum of 5.0-5.5). All three proteases are glycoproteins with molecular weight of about 70 kDa. Endogenous PC1/3 and PC2 showed similar substrate specificity and protease inhibitor profiles. Z-Arg-Val-Arg-Arg-MCA was the best substrate among fluorogenic tri- and tetrapeptides tested, with K_m values of 40 and 66 mM, and V_{max} values of 352 and 255 nmol/h/mg, for PC2 and PC1/3, respectively. Specificity of PC1/3 and PC2 for dibasic sites was confirmed by potent inhibition with active site-directed inhibitors (D-Tyr)-Glu-Phe-Lys-Arg-CH_2Cl and Ac-Arg-Arg-CH_2Cl. CG PC1/3 and PC2 were activated by DDT and inhibited by thiol-blocking reagents. PC1/3 and PC2 are calcium-dependent proteases that are inhibited by EGTA and stimulated by calcium. PC activities were reduced by several serpin inhibitors -- α_1-antitrypsin, α_1-antichymotrypsin (ACT), and ACT-like proteins from adrenal medulla. The 70 kDa aspartic protease from CG is sensitive to pepstatin A, a group specific inhibitor of aspartic proteinases, but not to inhibitors of serine, cysteine, and metallo-proteinases, and is activated by DTT. The aspartic protease cleaved recombinant proenkephalin between Lys_{-1}-Arg_{174}, located at the COOH-terminus of (Met)enkephalin-Arg-Gly-Leu. The similarity of this CG aspartic protease to other paired basic residue specific aspartic proteases -- pituitary POMC-converting enzyme and yeast aspartic proteinase 3 (YAP3) -- indicates CG 70 kDa aspartic proteinase as a member of the family of the candidate prohormone processing aspartic proteinases.

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